Seed-set and pollen–stigma compatibility in *Leymus chinensis*

W. D. Zhang*, S. Y. Chen*, G. S. Liu* and C. C. Jan†

*Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing, China, and †USDA-ARS, Northern Crop Science Laboratory, Fargo, ND, USA

Abstract

Chinese leymus [*Leymus chinensis* (Trin.) Tzvel.] is an important forage distributed in East Asia. The seed-set rates and the pollen–stigma compatibility in six populations were investigated in 2001. Proportionately seed-set ranged from 0·065 to 0·567 under open pollination and 0·0056 to 0·0426 under self-pollination. The former is significantly higher than the latter in each population. Microscopic observations showed that proportionately only 0·0551 to 0·1167 of self-pollinated pollen grains were compatible but most cross-pollinated pollen grains were compatible. The tubes of most incompatible pollen grains aborted upon entering into the stigmas. Among the six populations, there was a significant correlation between seed-set under open pollination and the compatible pollen rates under cross-pollination. These results suggest that Chinese leymus is a self-incompatible species, and the compatibility of pollen and stigma might be one of the factors influencing seed-set in natural conditions. This information will be useful for future breeding efforts.

Keywords: *Leymus chinensis*, seed-set, compatible pollen, pollination, self-incompatibility

Introduction

Chinese leymus [*Leymus chinensis* (Trin.) Tzvel., syn. *Aneurolepidium chinensis* (Trin.) Kitag. ($2n = 4x = 28$)] is a perennial species of Poaceae distributed widely in East Asia, including China, Japan, Mongolia and eastern Russia (Lu *et al.*, 1987). The grass is about 30–140 cm tall and has strong rhizomes (Anonymous, 1976). It is a cool-season grass found on arid and alkaline soils (Clayton and Renvoize, 1999). Its high production and nutritive value make the species important for livestock in north-eastern China (Wang *et al.*, 1997). Owing to the environmental problems associated with overstocking livestock on grasslands, there is an increasing demand for a grass species to restore and prevent further deterioration of natural grasslands (Li and Chen, 1997). The grasses in Poaceae display a range of breeding systems from dioecism to autonomous apomixes, and there exists different reproductive systems even in the same genus (Connor, 1979). It is necessary to understand about the reproductive system of Chinese leymus from a breeding and seed production standpoint. However, little information on the sexual reproduction of this forage is available. It had been reported that proportionately above 0·92 of pollen grains in mature anthers are viable and that microspore development does not hinder the seed-set of the grass (Ma *et al.*, 1984). However, the question of whether Chinese leymus is a self-incompatible forage has not been previously addressed. This paper describes seed-set and pollen–stigma compatibility under self- and cross-pollination.

Correspondence to: G. S. Liu, Key Laboratory of Photosynthesis and Environmental Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.
E-mail: liugs@ns.ibcas.ac.cn

Received 16 July 2003; revised 16 January 2004

Materials and methods

Materials

Six distinct populations were selected for these experiments. They were collected from various biotypes in north-eastern China (Table 1). In 2001, the seeds were sown in six plots in the Botanic Garden at the Institute of Botany, Chinese Academy of Sciences, Beijing, China (39°50′N, 116°20′E). At the site the mean annual temperature is 11·5°C. Mean temperature in January is –4·6°C and in July is 25·8°C. Annual precipitation is 644 mm. The period of frosts is from 12 October to 18 April.

Seed-set under field conditions

Seed-set studies were carried out in 2001. For each population, thirty plants were selected to represent the normal variation in their biological characters. Ten to

Table 1 Geographical and morphological descriptions of the six populations of *Leymus chinensis* used in the study.

Population	Sources	Main morphological characteristics
1	Haidian, Beijing 39°57′N 116°20′E	Yellow-green leaves, tall straw, early-flowering, about 220 florets per inflorescence
2	Yanqing, Beijing 40°25′N 115°58′E	Yellow-green leaves, intermediate height of slender straw, intermediate flowering, 140 florets per inflorescence
3	Duolun, Inner Mongolia Autonomous Region 42°36′ N 116°12′E	Grey-green leaves, tall and thick straw with strong resistance to lodging, 160 florets per inflorescence
4	Xilinhot, Inner Mongolia Autonomous Region 44°21′ N 126°19′E	Dark-green leaves, tall straw with the longest spike and most florets, late flowering, 240 florets per inflorescence
5	Harbin, Heilongjiang Province 45°5N 126°44′E	Deep-green leaves, intermediate height of straw and flowering, short spike, 130 florets per inflorescence
6	Qiqihaer, Heilongjiang Province 48°16′N 123°57′E	Short straw, late flowering, short spike with the least spikelets, about 100 florets per inflorescence

twelve spikes were selected from each plant; half of the spikes were open pollinated and the rest were enclosed within a 30 cm × 10 cm paper bag before anthesis for self-pollination. The bags were tightened and attached to stakes to support the spikes. The spikes were agitated daily to improve the pollen dispersal.

Seed-set rates under open and self-pollination were calculated by dividing the number of seeds by the number of florets. The numbers of florets were estimated by counting. Seeds were counted irrespective of their size, and seed-set was expressed on a proportional basis.

Compatible pollen rate on stigma

Ten typical plants were sampled in each population. Pollinations were performed *in vitro* using the technique of Lundqvist (1961). Pistils from four to five randomly selected plants were carefully removed from florets and transferred into a Petri dish. A thin cloud of fresh pollen collected from one of the plants was released over the stigmas. This resulted in self-pollination and three to four cross-pollination combinations in each Petri dish. Pistils of all the ten plants were self-pollinated in the same way. Pollinated stigmas were incubated at room temperature (22–25°C) for 24 h. Thereafter, the stigmas were placed on a glass slide in a drop of aniline blue in lactophenol to observe the pollen under a light microscope (Hayman, 1956; Weimarck, 1968). Compatible pollen grains were stained faintly whereas the incompatible grains were stained darkly (Figure 1).

The compatible pollen rate in each combination was calculated by dividing the number of compatible pollen grains by the total number of pollen grains. Pollen tubes were examined by means of the callose fluorochrome reaction (Lalouette, 1967; Cornish *et al.*, 1979a,b). Photomicrographs were taken with a Leica MPS32 (Leica, Wetzlar, Germany) microscope.

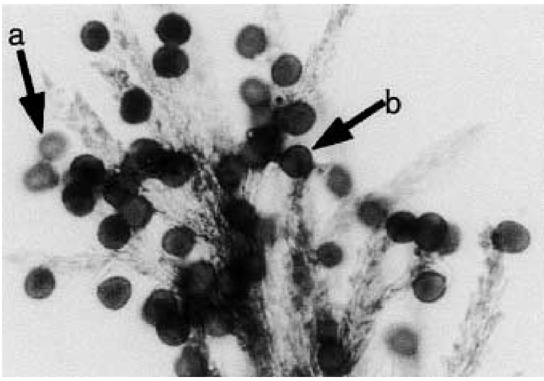

Figure 1 Photomicrograph of compatible [stained faintly, indicated with arrow (a)] and incompatible [stained darkly, indicated with arrow (b)] pollens of *Leymus chinensis* on the stigma under light microscopy (250×).

Analysis of data

As values representing the rates of seed-set and compatible pollen were of a low or high order of magnitude, the arc-sine transformation was employed for all data expressed as proportions in the statistical analysis. All data were analysed using the SPSS program (SPSS, 2000). To detect significant differences within each population, a multiple comparison (Student–Newman–Keuls) test was performed. Associations were expressed as Pearson correlation coefficients (two-tailed).

Results

Description of anthesis

The inflorescence of Chinese leymus is a compact spike. The period of anthesis was not identical for the different populations in Beijing. The flowering time of all populations lasted for 2 weeks in the middle of May but for a specific population the time of anthesis lasted only for 8 d at a maximum. The most profuse flowering occurred from the third to the sixth day. The stigmas emerge from the florets simultaneously with anther extension. The scattering of pollen on stigmas is abundant. The distance between stigma and anther was about 2–4 mm (Figure 2). The pollen was carried easily in the wind under field conditions. Hence, both self- and open-pollination could occur without obstacle during pollination.

Seed-set

All six populations produced seeds upon open pollination, with a seed-set range of 0·064–0·554 (Table 2). Three groups could be distinguished in terms of seed-set rate. Population 1 had the highest proportion at 0·554,

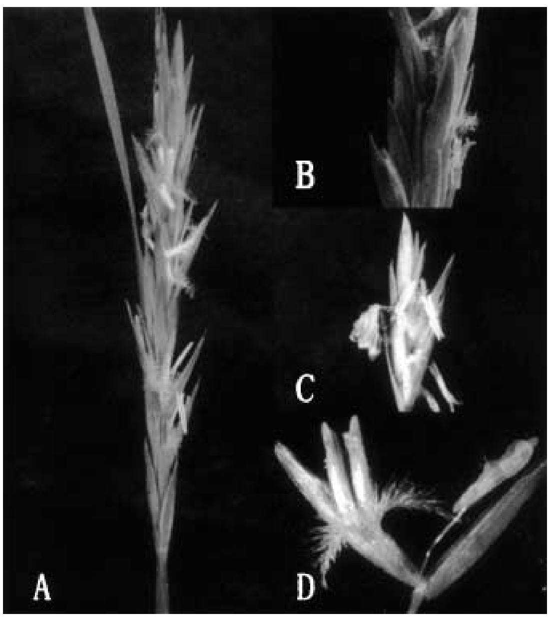

Figure 2 Spike, spikelets and florets of *Leymus chinensis*. (A) Mature stigmas and anthers exposing themselves at the same time in a spike (the spike is about 8·5 cm long); (B) withered stigmas and anthers having spread out most of their pollen after anthesis in the spike; (C) dissected spikelet with the withered stigmas and dehisced anthers; and (D) detached floret with branched stigmas, three anthers between palea and lemma.

Table 2 Seed-set rates under self- and open-pollination in the six populations of *Leymus chinensis* (mean with standard deviations of the mean in parentheses).

Population	Self-pollination	Open pollination
1	0·029 (0·0065)	0·554 (0·1126)
2	0·031 (0·0099)	0·417 (0·1008)
3	0·006 (0·0018)	0·066 (0·0141)
4	0·005 (0·0016)	0·354 (0·1078)
5	0·044 (0·0124)	0·441 (0·0976)
6	0·001 (0·0003)	0·093 (0·0197)

Significant at $P < 0·01$.

while population 3 had the lowest at 0·064, and populations 2, 4, 5 and 6 were intermediate. However, all populations had very poor seed-set under self-pollination, from 0·0130 to 0·0436. Analysis of variance indicated that differences in seed-set between self- and open-pollination were significant in each population ($P < 0·001$).

Under self-pollination, the six populations could be divided into four groups: group A (population 5), group B (populations 1 and 2), group C (populations 3 and 4), and group D (population 6) respectively. They were different at the $P < 0·01$ level of significance. Populations 3 and 4 had different values for seed-set at the $P < 0·05$ level of significance. Under open pollination, the six populations could also be divided into four groups: group A (population 1), group B (populations 4 and 5), group C (population 2) and group D (populations 3 and 6) respectively. They were different at the $P < 0·01$ level of significance. Populations 3 and 6 were different for seed-set at the $P < 0·05$ level of significance.

Compatible pollen rates on stigma

In the six populations, mean compatible pollen rates were 0·0551–0·1167 under self-pollination and 0·6000–0·8483 under cross-pollination. Analysis of variance indicated a significant difference in compatible pollen rates between self- and cross-pollination in each population ($P < 0·001$) (Table 3).

Under self-pollination, there was no significant difference in the proportion of compatible pollen grains among the six populations ($P > 0·05$). Under cross-pollination, the six populations could be divided into three groups: group A (population 1), group B (populations 2, 4 and 5) and group C (populations 3 and 6) respectively. They were different for compatible pollen rates at the $P < 0·01$ level of significance.

Under a fluorescence microscope, it could be observed that most pollen had no difficulty in entering the stigma but the most incompatible pollens were

Table 3 Compatible pollen rates under self- and cross-pollination in each population of *Leymus chinensis* (mean with standard deviations of mean in parentheses).

Population	Self-pollination	Cross-pollination
1	0·055 (0·0302)	0·848 (0·0894)
2	0·091 (0·0414)	0·720 (0·1030)
3	0·081 (0·0370)	0·634 (0·1305)
4	0·067 (0·0423)	0·768 (0·0586)
5	0·117 (0·0770)	0·722 (0·0860)
6	0·096 (0·0435)	0·600 (0·1039)

Significant at $P < 0{\cdot}01$.

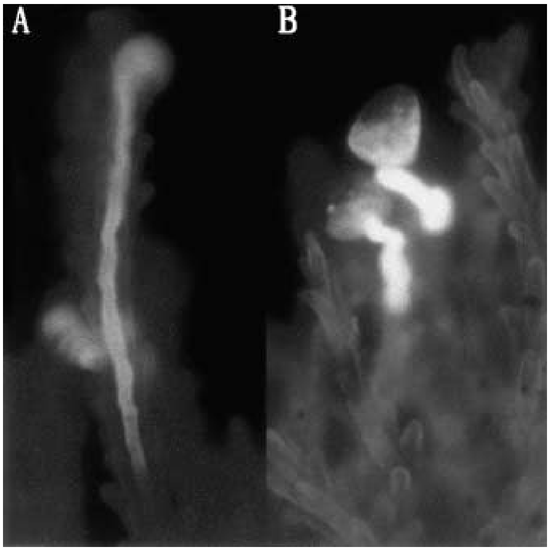

Figure 3 Pollen tubes under fluorescence microscopy (500×). (A) Compatible pollen tube penetrating into the stigmas. (B) The incompatible pollen usually arrested after the tubes have just penetrated into the stigma.

blocked immediately. Their tubes were bright, short and swollen, and some of them even twisted at the end. The compatible pollen tubes went deeply down into the stigma (Figures 3 and 4).

Correlation analysis

The correlation between seed-set under self- and open-pollination was not significant ($P < 0{\cdot}05$). Because there was no significant difference between compatible pollen rates under self-pollination among the six populations ($P > 0{\cdot}05$), further analyses were not performed. However, the correlation ($r = 0{\cdot}91$) between seed-set rates under open pollination and compatible pollen rates under cross-pollination was significant at the $P < 0{\cdot}05$ level (two-tailed test).

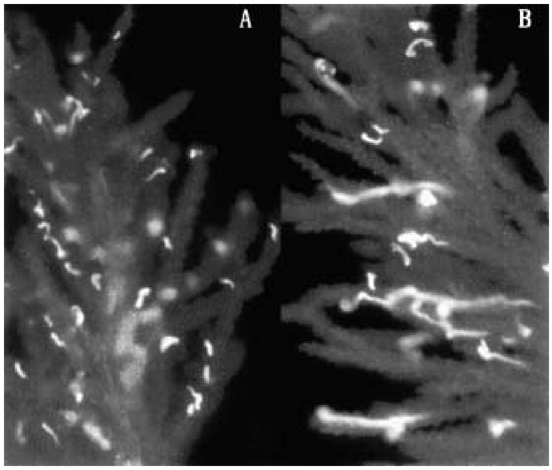

Figure 4 Fluorescence micrograph showing pollen tubes penetrating into or arrested on stigma (500×). Most pollen grains are (A) incompatible or (B) compatible.

Discussion

Self-incompatible flowering plants, while possessing male and female gametes, fail to form zygotes after self-pollination but succeed after cross-pollination with certain other plants of the same species. Self-incompatible and self-compatible plants are typically found in the same genus, and the frequency of self-incompatibility is higher in perennial than annual species (Beddows, 1930). The grass family contains 650–700 genera and includes *c.* 10 000 species (Watson and Dallwitz, 1992). Self-incompatibility is present in at least forty-nine genera (Connor, 1979; Baumann *et al.*, 2000). Genetic control of self-incompatibility has been investigated in several genera important in forage science, including the genera containing *Dactylis*, *Festuca*, *Lolium* and *Phalaris* species (de Nettancourt, 1993). Lundqvist (1956) was the first to describe that gametophytic self-incompatibility in the diploid grasses was genetically controlled by two unlinked and multi-allelic loci, *S* and *Z*. Based on observations of pollen development on the stigma, Cornish *et al.* (1979a,b) concluded that self-incompatibility in *Lolium perenne* is also controlled by a pair of independently inherited, multi-allelic genes whose effect on pollen is gametophytic, and later the conclusion was examined for seed-set in diploid *L. perenne* (Cornish *et al.*, 1980). To utilize the two-locus incompatibility system for the production of F_1 hybrids, schemes were proposed for producing tetraploid populations with a high level of incompatibility within a population or by using single- or double-cross hybrids from the dihaploids (Hayward, 1988). However, F_1 hybrids have been developed only in the limited range of grass forage species in the world whose mechanisms for controlling cross-pollination are available (Hayward, 1988).

The present investigation deals with seed-set and pollen–stigma compatibility in Chinese leymus, which is a predominant forage in eastern Asia. In all six populations of Chinese leymus, there was no temporal or spatial isolation between the extension of stigmas and the pollen disposal of anthers. Self-pollination resulted in low seed-set, and open pollination resulted in significantly high seed-set. The proportion of compatible pollen under self-pollination was much lower than under cross-pollination. These results indicate that *Leymus chinensis* is a cross-pollinated species. This mechanism of reproduction is similar to that of most perennial grasses, which are nearly all cross-pollinated (Poehlman, 1979).

In order to establish the mode of genetic control in grasses, Hayman (1956) developed a technique to distinguish the compatible and incompatible pollens on stigmas. This method was effective in determining whether a grass was self-compatible (Weimarck, 1968). There are no reports on the correlation between seed-set and compatible pollen rates among different populations. Our results indicated a significant correlation between seed-set under open pollination and compatible pollen under cross-pollination.

Our results also showed that there was no correlation between self- and open-pollination in seed-set ($P > 0{\cdot}05$). In addition, among the six populations, compatible pollen rates were not significantly different but seed-set under self-pollination was different at the $P < 0{\cdot}01$ level of significance. These results suggest that compatibility of pollen stigma might be one of the factors influencing seed-set under open pollination. Pollen tube growth, zygotic formation, zygotic inviability, embryo abortion and endosperm abnormalities should also be considered in a population's seed-set, especially under self-pollination (Falcinelli, 1999).

The tube growth of self pollen was blocked frequently on the surface of stigma in Chinese leymus, which is similar to that of *Hordeum bulbosum* ($2n = 14$) but different from *Gaudinia fragilis* ($2n = 14$) (Heslop-Harrison, 1982). Both of these grasses are self-incompatible. In addition, autotetraploid *L. perenne* is self-incompatible, as is the diploids from which they have been induced, and only one *S-Z* pair of the pollen of tetraploids needs to be matched in the stigma for incompatibility to occur (Fearon *et al.*, 1984a,b). Chinese leymus is allotetraploid with chromosome karyotype NsNsXmXm (Duan and Fan, 1984). While at least one Psathyrostachys genome (Ns) has been substantiated in *L. chinensis* (Zhang and Dvorak, 1991; Wang and Jensen, 1994; Wang *et al.*, 1994; Anamthawat-Jónsson and Bödvarsdóttir, 2001), the consensus of several researchers has been to reserve designation of another Leymus genome, Xm, as unknown (Wang and Jensen, 1994; Wang *et al.*, 1994; Sun *et al.*, 1995). Therefore, the pollen–stigma interaction and genetic control in Chinese leymus certainly needs further investigation.

Self-incompatibility can be exploited for the production of hybrids. The Chinese leymus evaluated in this paper suggests future potential because the high self-incompatibility significantly reduces hand emasculation. However, more information regarding heterosis and the efficiency of seed production by means of self-incompatibility is needed.

Acknowledgments

Our special thanks are due to our co-workers Qingyan Shu, Fangfang Li and Guijun Dong for their contribution to this research. The authors also wish to thank L. G. Campbell and B. A. Vick for their critical review of the manuscript. This research is part of the Biotechnology of Pasture Plant Program that is funded by the Key Project of the Chinese Academy of Sciences (NK '10.5'. A-05) and the Ministry of Science and Technology, China (2001BA707B02).

References

Anamthawat-Jónsson K. and Bödvarsdóttir S. (2001) Genomic and genetic relationships among species of *Leymus* (Poaceae: Triticeae) inferred from 18S – 26S ribosomal genes. *American Journal of Botany*, **88**, 553–559.

Anonymous (1976) *Aneurolepidium chinensis*. In: Institute of Botany, The Chinese Academy of Sciences (ed.) *Iconographia Cormophytorum Sinicorum, Tomus*, pp. 1–87. Beijing, China: Science Press.

Baumann U., Juttner J., Bian X. and Langridge P. (2000) Self-incompatibility in the grasses. *Annal of Botany*, **85**(Suppl. A), 203–209.

Beddows A.R. (1930) Seed setting and flowering in various grasses. *University College of Wales Series H*, **12**, 5–99.

Clayton W.D. and Renvoize S.A. (1999) *Genera Graminum, Grasses of the World. Kew Bulletin Additional Series XIII*. Royal Botanic Gardens, Kew, London: The Board of Trustees of the Royal Gardens, Kew.

Connor H.E. (1979) Breeding systems in the grasses: a survey. *New Zealand Journal of Botany*, **17**, 547–574.

Cornish M.A., Hayward M.D. and Lawrence M.J. (1979a) Self-incompatibility in ryegrass. I. Genetic control in diploid *Lolium perenne* L. *Heredity*, **43**, 95–106.

Cornish M.A., Hayward M.D. and Lawrence M.J. (1979b) Self-incompatibility in ryegrass. II. The joint segregation of *S* and *Z* in *Lolium perenne* L. *Heredity*, **43**, 129–136.

Cornish M.A., Hayward M.D. and Lawrence M.J. (1980) Self-incompatibility in ryegrass. IV. Seed-set in diploid *Lolium perenne* L. *Heredity*, **44**, 333–340.

Duan X.G. and Fan J.L. (1984) Studies on the chromosome karyotype of Chinese Leymus. *Grassland of China*, **1**, 63–65.

FALCINELLI M. (1999) Temperate forage seed production: conventional and potential breeding strategies. *Journal of New Seeds*, **1**, 37–66.

FEARON C.H., HAYWARD M.D. and LAWRENCE M.J. (1984a) Self-incompatibility in ryegrass. VII. The determination of incompatibility genotypes in autotetraploid families of *Lolium perenne* L. *Heredity*, **53**, 403–413.

FEARON C.H., HAYWARD M.D. and LAWRENCE M.J. (1984b) Self-incompatibility in ryegrass. VIII. The mode of action of *S* and *Z* alleles in the pollen of autotetraploids of *Lolium perenne* L. *Heredity*, **53**, 415–422.

HAYMAN D.L. (1956) The genetic control of incompatibility in *Phalaris coerulescens* Desf. *Australian Journal of Biological Sciences*, **9**, 321–331.

HAYWARD M.D. (1988) Exploitation of the incompatibility mechanism for the production of F_1 hybrid forage grasses. *Euphytica*, **39**, 33–37.

HESLOP-HARRISON J. (1982) Pollen–stigma interaction and cross-incompatibility in the grasses. *Science*, **215**, 1358–1364.

LALOUETTE J.A. (1967) Growth of grass pollen tubes exhibited by callose fluorochrome reaction. *Grana Palynologica*, **7**, 2–3.

LI L. and CHEN Z. (1997) Changes in soil carbon storage due to over-grazing in *Leymus chinensis* steppe in the Xilin River Basin of Inner Mongolia. *Journal of Environmental Sciences*, **4**, 486–490.

LU S., SUN Y. and LIU S. (1987) *Flora Peipublicae Popularis Sinica. Tomus 9 (30)*. Beijing: Science Press.

LUNDQVIST A. (1956) Self-incompatibility in rye. I. Genetic control in the diploid. *Hereditas*, **42**, 293–348.

LUNDQVIST A. (1961) A rapid method for the analysis of incompatibilities in grasses. *Hereditas*, **47**, 705–707.

MA H., YUAN T. and WANG F. (1984) The characterization of set and the reason for low seed-set percentage in *Leymus chinensis*. *Grassland of China*, **3**, 15–20.

DE NETTANCOURT D. (1993) Self- and cross-incompatibility system. In: Hayward M.D., Bosemark N.O. and Romagosa I. (eds) *Plant Breeding*, pp. 203–212. London, UK: Chapman & Hall.

POEHLMAN J.M. (1979) *Breeding Field Crops*, 2nd edn. Westport, CT, USA: AVI Publishing Company, Inc.

SPSS (2000) *SPSS for Windows, Release 10.0*. Chicago, IL, USA: SPSS.

SUN G.L., YEN C. and YANG J.L. (1995) Morphology and cytology of intergenetic hybrids involving *Leymus multicaulis* (Poaceae). *Plant Systematics and Evolution* **194**, 83–91.

WANG R.R.-C. and JENSEN K.B. (1994) Absence of the J genome in *Leymus* species (Poaceae: Triticeae): evidence from DNA hybridization and meiotic pairing. *Genome*, **37**, 231–235.

WANG R.R.-C., VON BOTHMER R., DVORAK J., LINDE-LAURSEN I. and MURAMATSU M. (1994) Genome symbols in the Triticeae (Poaceae). In: Wang R.R.-C., Jensen K.B. and Junnsi C. (eds) *Proceedings of the 2nd International Triticeae Symposium, Logan, Utah, 20–24 June 1994*, pp. 29–31. Logan, UT, USA: Utah State University Press.

WANG R., RIPLEY J.B. and YANG H. (1997) Effects of grazing of *Leymus chinensis* on the Songnen Plain of northeastern China. *Journal of Arid Environments*, **36**, 307–318.

WATSON L. and DALLWITZ M.Z. (1992) *The Grass Genera of the World*. Wallingford: CAB International.

WEIMARCK A. (1968) Self-incompatibility in the Gramineae. *Hereditas*, **60**, 157–166.

ZHANG H.B. and DVORAK J. (1991) The genome origin of tetraploid species of *Leymus* (Poaceae: Triticeae) inferred from variation in repeated nucleotide sequences. *American Journal of Botany*, **78**, 871–884.